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FILE 'BIOSIS' ENTERED AT 14:13:18 ON 19 JUL 2002
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=> S FPN1
L1            8 FPN1

=> S L1 AND HUMAN
L2            5 L1 AND HUMAN

=> D 1

L2   ANSWER 1 OF 5     MEDLINE
AN   2002153036     MEDLINE
DN   21830233   PubMed ID: 11842003
TI   Copper repletion enhances apical iron uptake and transepithelial iron
     transport by Caco-2 cells.
AU   Han Okhee; Wessling-Resnick Marianne
CS   Department of Nutrition, Harvard School of Public Health, Boston, MA
     02115, USA.
NC   DK-40561 (NIDDK)
     DK-55495 (NIDDK)
SO   AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY,
     (2002 Mar) 282 (3) G527-33.
     Journal code: 100901227. ISSN: 0193-1857.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200203
ED   Entered STN: 20020312
     Last Updated on STN: 20020403
     Entered Medline: 20020327


=> D 2

L2   ANSWER 2 OF 5     MEDLINE
AN   2001567204     MEDLINE
DN   21527003   PubMed ID: 11673399
TI   Recent advances in disorders of iron metabolism: mutations, mechanisms and
     modifiers.
AU   Roy C N; Andrews N C
CS   Division of Hematology/Oncology, Children's Hospital, Department of
     Pediatrics, Harvard Medical School, Boston, MA 02115, USA.
NC   T32-HL07623-15 (NHLBI)
SO   HUMAN MOLECULAR GENETICS, (2001 Oct 1) 10 (20) 2181-6.  Ref: 62
     Journal code: 9208958. ISSN: 0964-6906.
CY   England: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
     General Review; (REVIEW)
     (REVIEW, TUTORIAL)
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 NEWS  4  Feb 01   DKILIT now produced by FIZ Karlsruhe and has a new update
                   frequency
 NEWS  5  Feb 19   Access via Tymnet and SprintNet Eliminated Effective 3/31/02
 NEWS  6  Mar 08   Gene Names now available in BIOSIS
 NEWS  7  Mar 22   TOXLIT no longer available
 NEWS  8  Mar 22   TRCTHERMO no longer available
 NEWS  9  Mar 28   US Provisional Priorities searched with P in CA/CAplus
                   and USPATFULL
 NEWS 10  Mar 28   LIPINSKI/CALC added for property searching in REGISTRY
 NEWS 11  Apr 02   PAPERCHEM no longer available on STN.  Use PAPERCHEM2 instead.
 NEWS 12  Apr 08   "Ask CAS" for self-help around the clock
 NEWS 13  Apr 09   BEILSTEIN: Reload and Implementation of a New Subject Area
 NEWS 14  Apr 09   ZDB will be removed from STN
 NEWS 15  Apr 19   US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
 NEWS 16  Apr 22   Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
 NEWS 17  Apr 22   BIOSIS Gene Names now available in TOXCENTER
 NEWS 18  Apr 22   Federal Research in Progress (FEDRIP) now available
 NEWS 19  Jun 03   New e-mail delivery for search results now available
 NEWS 20  Jun 10   MEDLINE Reload
 NEWS 21  Jun 10   PCTFULL has been reloaded
 NEWS 22  Jul 02   FOREGE no longer contains STANDARDS file segment
 NEWS 23  Jul 19   NTIS to be reloaded July 28, 2002

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               CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP),
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LA English
FS Priority Journals
EM 200201
ED Entered STN: 20011024
Last Updated on STN: 20020125
Entered Medline: 20020117

=> D 3

L2 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:354116 BIOSIS
DN PREV200200354116
TI Copper supplementation stimulates apical iron uptake and transepithelial transport in intestinal cells.
AU Han, Okhee (1); Wessling-Resnick, Marianne
CS (1) Nutritional Sciences, Oklahoma State University, HES 425, Stillwater, OK, 74078 USA
SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A375. http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
DT Conference
LA English

=> D 4

L2 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:238716 BIOSIS
DN PREV200200238716
TI Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells.
AU Han, Okhee; Wessling-Resnick, Marianne (1)
CS (1) Dept. of Nutrition, Harvard School of Public Health, 665 Huntington Ave., Boston, MA, 02115 USA
SO American Journal of Physiology, (March, 2002) Vol. 282, No. 3 Part 1, pp. G527-G533. http://www.ajpcon.org. print.
ISSN: 0002-9513.
DT Article
LA English

=> D 5

L2 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:526571 BIOSIS
DN PREV200100526571
TI Recent advances in disorders of iron metabolism: Mutations, mechanisms and modifiers.
AU Roy, Cindy N.; Andrews, Nancy C. (1)
CS (1) Division of Hematology, Children's Hospital, 300 Longwood Avenue, Enders 720, Boston, MA, 02115: nancy_andrews@hms.harvard.edu USA
SO Human Molecular Genetics, (1 October, 2001) Vol. 10, No. 20, pp. 2181-2186. print.
ISSN: 0964-6906.
DT General Review
LA English
SL English

=> D 6
5 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE

The answer numbers requested are not in the answer set.
ENTER ANSWER NUMBER OR RANGE (1):5

L2 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:526571 BIOSIS
DN PREV200100526571
TI Recent advances in disorders of iron metabolism: Mutations, mechanisms and modifiers.
AU Roy, Cindy N.; Andrews, Nancy C. (1)
CS (1) Division of Hematology, Children's Hospital, 300 Longwood Avenue, Enders 720, Boston, MA, 02115: nancy_andrews@hms.harvard.edu USA
SO Human Molecular Genetics, (1 October, 2001) Vol. 10, No. 20, pp. 2181-2186. print.
ISSN: 0964-6906.
DT General Review
LA English
SL English

=> S CACO CELLS
L3 28 CACO CELLS

=> DUP REM L3
PROCESSING COMPLETED FOR L3
L4 23 DUP REM L3 (5 DUPLICATES REMOVED)

=> S L4 AND IRON
L5 0 L4 AND IRON

=> S L4 AND TRANSPORTER
L6 2 L4 AND TRANSPORTER

=> D 1

L6 ANSWER 1 OF 2 MEDLINE
AN 93066280 MEDLINE
DN 93066280 PubMed ID: 1438248
TI Distribution of mRNA of a Na(+)-independent neutral amino acid **transporter** cloned from rat kidney and its expression in mammalian tissues and Xenopus laevis oocytes.
AU Yan N; Mosckovitz R; Udenfriend S; Tate S S
CS Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Nov 1) 89 (21) 9982-5.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199212
ED Entered STN: 19930122
Last Updated on STN: 19980206
Entered Medline: 19921211

=> D 2

L6 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:226874 BIOSIS
DN PREV199395118049
TI Expression of human intestinal dipeptide **transporter** in Xenopus laevis oocytes.
AU Saito, Hideyuki; Ishii, Tetsuro; Inui, Ken-Ichi (1)

CS (1) Dep. Hosp. Pharm., Sch. Med., Tokyo Med. Dental Univ., 1-5-45, Yushima, Bunkyo-ku, Tokyo 113 Japan
SO Biochemical Pharmacology, (1993) Vol. 45, No. 3, pp. 776-779. ISSN: 0006-2952.
DT Article
LA English

=> S FERROPORTIN AND TRANSPORTER AND HUMAN
L7 7 FERROPORTIN AND TRANSPORTER AND HUMAN

=> DUP REM L7
PROCESSING COMPLETED FOR L7
L8 5 DUP REM L7 (2 DUPLICATES REMOVED)

=> D 1

L8 ANSWER 1 OF 5 MEDLINE DUPLICATE 1
AN 2002153036 MEDLINE
DN 21830233 PubMed ID: 11842003
TI Copper repletion enhances apical iron uptake and transepithelial iron transport by Caco-2 cells.
AU Han Okhee; Wessling-Resnick Marianne
CS Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA.
NC DK-40561 (NIDDK)
DK-55495 (NIDDK)
SO AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY, (2002 Mar) 282 (3) G527-33.
Journal code: 100901227. ISSN: 0193-1857.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20020312
Last Updated on STN: 20020403
Entered Medline: 20020327

=> D 2

L8 ANSWER 2 OF 5 MEDLINE DUPLICATE 2
AN 2001262128 MEDLINE
DN 21213852 PubMed ID: 11313311
TI Expression of the duodenal iron transporters divalent-metal **transporter** 1 and **ferroportin** 1 in iron deficiency and iron overload.
AU Zoller H; Koch R O; Theurl I; Obrist P; Pietrangelo A; Montosi G; Haile D J; Vogel W; Weiss G
CS Department of Internal Medicine, Innsbruck, Austria.
SO GASTROENTEROLOGY, (2001 May) 120 (6) 1412-9.
Journal code: 0374630. ISSN: 0016-5085.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200105
ED Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

=> D 3

L8 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:264055 BIOSIS
DN PREV200100264055
TI Utilization of the zebrafish to understand hematopoiesis.
AU Bahary, Nathan (1); Zon, Leonard I. (1)
CS (1) Hematology/Oncology, Children's Hospital, 300 Longwood Ave, Ender's 720, Boston, MA, 02115 USA
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1102. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DT Conference
LA English
SL English

=> D 4

L8 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:526025 BIOSIS
DN PREV200100526025
TI Duodenal expression of **ferroportin** 1 in patients with hereditary haemochromatosis and iron deficiency.
AU Ryan, E. (1); Byrnes, V. (1); Kelleher, B. (1); Barrett, S. (1); O'Keane, J. C.; Crowe, J.
CS (1) Centre for Liver Disease, Dublin Ireland
SO Hepatology, (October, 2001) Vol. 34, No. 4 Pt. 2, pp. 208A. print.
Meeting Info.: 52nd Annual Meeting and Postgraduate Courses of the American Association for the Study of Liver Diseases Dallas, Texas, USA November 09-13, 2001
ISSN: 0270-9139.
DT Conference
LA English
SL English

=> D 5

L8 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:50378 BIOSIS
DN PREV200100050378
TI Membranes iron transport: New insights.
Original Title: Du nouveau dans le transport membranaire du fer..
AU Borensztein, Pascale (1)
CS (1) Medecine/Sciences, Inserm U.474, Hopital Port-Royal, 123, Boulevard de Port-Royal, 75014, Paris France
SO M-S (Medecine Sciences), (Juin Juillet, 2000) Vol. 16, No. 6-7, pp. 833-835. print.
ISSN: 0767-0974.
DT Article
LA French
SL English

=> FIL STNGUIDE

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	19.14	19.35

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	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	1.44	20.79

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=> S IRON AND TRANSPORTER
L9 971 IRON AND TRANSPORTER

=> DUP REM L9
PROCESSING COMPLETED FOR L9
L10 546 DUP REM L9 (425 DUPLICATES REMOVED)

=> S L10 AND HUMAN
L11 187 L10 AND HUMAN

=> S L11 AND FERROPORTIN
L12 5 L11 AND FERROPORTIN

=> S L11 AND FERROPORTIN?
L13 10 L11 AND FERROPORTIN?

=> D 1

L13 ANSWER 1 OF 10 MEDLINE
AN 2002223758 MEDLINE
DN 21922921 PubMed ID: 11925462
TI **Iron** treatment downregulates DMT1 and IREG1 mRNA expression in Caco-2 cells.
AU Martini Ligia A; Tchack Laurie; Wood Richard J
CS Mineral Bioavailability Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA.
SO JOURNAL OF NUTRITION, (2002 Apr) 132 (4) 693-6.
Journal code: 0404243. ISSN: 0022-3166.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20020419
Last Updated on STN: 20020429
Entered Medline: 20020426

=> D 2

L13 ANSWER 2 OF 10 MEDLINE
AN 2002153036 MEDLINE
DN 21830233 PubMed ID: 11842003
TI Copper repletion enhances apical **iron** uptake and transepithelial

iron transport by Caco-2 cells.
AU Han Okhee; Wessling-Resnick Marianne
CS Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA.
NC DK-40561 (NIDDK)
DK-55495 (NIDDK)
SO AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY, (2002 Mar) 282 (3) G527-33.
Journal code: 100901227. ISSN: 0193-1857.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20020312
Last Updated on STN: 20020403
Entered Medline: 20020327

=> D 3

L13 ANSWER 3 OF 10 MEDLINE
AN 2001262128 MEDLINE
DN 21213852 PubMed ID: 11313311
TI Expression of the duodenal **iron** transporters divalent-metal **transporter** 1 and **ferroportin** 1 in **iron** deficiency and **iron** overload.
AU Zoller H; Koch R O; Theurl I; Obrist P; Pietrangelo A; Montosi G; Haile D J; Vogel W; Weiss G
CS Department of Internal Medicine, Innsbruck, Austria.
SO GASTROENTEROLOGY, (2001 May) 120 (6) 1412-9.
Journal code: 0374630. ISSN: 0016-5085.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200105
ED Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

=> D 4

L13 ANSWER 4 OF 10 MEDLINE
AN 2001085911 MEDLINE
DN 20563914 PubMed ID: 11110669
TI **Iron** homeostasis: new tales from the crypt.
AU Roy C N; Enns C A
CS Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, OR 97201-3098, USA.
NC DK 54488 (NIDDK)
T32-HL00781 (NHLBI)
SO BLOOD, (2000 Dec 15) 96 (13) 4020-7. Ref: 119
Journal code: 7603509. ISSN: 0006-4971.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200101
ED Entered STN: 20010322
Last Updated on STN: 20010322

Entered Medline: 20010118

=> D 5

L13 ANSWER 5 OF 10 MEDLINE
AN 2001033218 MEDLINE
DN 20461127 PubMed ID: 11005792
TI Haemochromatosis: novel gene discovery and the molecular pathophysiology of **iron** metabolism.
AU Griffiths W; Cox T
CS Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK.
SO HUMAN MOLECULAR GENETICS, (2000 Oct) 9 (16) 2377-82. Ref: 37
Journal code: 9208958. ISSN: 0964-6906.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200011
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001130

=> D 6

L13 ANSWER 6 OF 10 MEDLINE
AN 2000155474 MEDLINE
DN 20155474 PubMed ID: 10693807
TI Positional cloning of zebrafish **ferroportin1** identifies a conserved vertebrate **iron** exporter.
CM Comment in: Nature. 2000 Feb 17;403(6771):711, 713
AU Donovan A; Brownlie A; Zhou Y; Shepard J; Pratt S J; Moynihan J; Paw B H; Drejer A; Barut B; Zapata A; Law T C; Brugnara C; Lux S E; Pinkus G S; Pinkus J L; Kingsley P D; Palis J; Fleming M D; Andrews N C; Zon L I
CS Department of Medicine, Children's Hospital and Dana-Farber Cancer Institute, Boston, Massachusetts 02115, USA.
SO NATURE, (2000 Feb 17) 403 (6771) 776-81.
Journal code: 0410462. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AA226612; GENBANK-AA226613; GENBANK-AA226614
EM 200003
ED Entered STN: 20000330
Last Updated on STN: 20000330
Entered Medline: 20000320

=> D 7

L13 ANSWER 7 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:222056 BIOSIS
DN PREV200200222056
TI Pathways for the regulation of DMT-1 and FP-1 expression by **iron** in **human** intestine.
AU Zoller, Heinz (1); Theurl, Igor (1); Koch, Robert (1); Vogel, Wolfgang (1); Weiss, Guenter (1)
CS (1) Univ, Innsbruck, Innsbruck Austria
SO Gastroenterology, (April, 2001) Vol. 120, No. 5 Supplement 1, pp. A.680.

http://www.gastrojournal.org/. print.
Meeting Info.: 102nd Annual Meeting of the American Gastroenterological Association and Digestive Disease Week Atlanta, Georgia, USA May 20-23, 2001
ISSN: 0016-5085.
DT Conference
LA English

=> D 8

L13 ANSWER 8 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:526025 BIOSIS
DN PREV200100526025
TI Duodenal expression of **ferroportin** 1 in patients with hereditary haemochromatosis and **iron** deficiency.
AU Ryan, E. (1); Byrnes, V. (1); Kelleher, B. (1); Barrett, S. (1); O'Keane, J. C.; Crowe, J.
CS (1) Centre for Liver Disease, Dublin Ireland
SO Hepatology, (October, 2001) Vol. 34, No. 4 Pt. 2, pp. 208A. print.
Meeting Info.: 52nd Annual Meeting and Postgraduate Courses of the American Association for the Study of Liver Diseases Dallas, Texas, USA November 09-13, 2001
ISSN: 0270-9139.
DT Conference
LA English
SL English

=> D 9

L13 ANSWER 9 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:264055 BIOSIS
DN PREV200100264055
TI Utilization of the zebrafish to understand hematopoiesis.
AU Bahary, Nathan (1); Zon, Leonard I. (1)
CS (1) Hematology/Oncology, Children's Hospital, 300 Longwood Ave, Ender's 720, Boston, MA, 02115 USA
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1102. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DT Conference
LA English
SL English

=> D 10

L13 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:50378 BIOSIS
DN PREV200100050378
TI Membranes **iron** transport: New insights.
Original Title: Du nouveau dans le transport membranaire du fer..
AU Borensztein, Pascale (1)
CS (1) Medecine/Sciences, Inserm U.474, Hopital Port-Royal, 123, Boulevard de Port-Royal, 75014, Paris France
SO M-S (Medecine Sciences), (Juin Juillet, 2000) Vol. 16, No. 6-7, pp. 833-835. print.
ISSN: 0767-0974.
DT Article
LA French
SL English

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=> FIL STNGUIDE
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     11.91      32.70

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LAST RELOADED: Jul  5, 2002 (20020705/UP).

=> LOGOFF HOLD
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                      0.60      33.30

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 14:42:12 ON 19 JUL 2002
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STIC-ILL

QH 301. F4

Thdc

Fr m: Wegert, Sandra
Sent: Friday, July 19, 2002 2:32 PM
To: STIC-ILL
Subject: ILL 09715927

PLEASE OBTAIN THE FOLLOWING REFERENCE:
THANKS A LOT

Utilization of the zebrafish to understand hematopoiesis.
AU Bahary, Nathan (1); Zon, Leonard I. (1)
CS (1) Hematology/Oncology, Children's Hospital, 300 Longwood Ave, Ender's 720, Boston, MA, 02115 USA
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1102. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.

Sandra Wegert
CM1 10D12
308-9346
AU 1647
Mailbox 10C01

857.1

Nicotine Inhibits Epithelial-to-Mesenchymal Transformation during Murine Palate Fusion

PEI KANG, JAN LALOR, ADRIENNE DOUGLAS, KATHY K. H. SVOBODA: BAYLOR COLLEGE OF DENTISTRY, TEXAS A&M UNIVERSITY SYSTEM, 3302 GASTON AVE, DALLAS, TX 75266-0677

Maternal smoking has been suggested by epidemiological studies as a risk factor for cleft palate. There is evidence that nicotine regulates cell migration. Epithelial-to-mesenchymal transformation is a key mechanism for palatal fusion. During this transition, medial edge epithelia from both palatal shelves lose cell-cell adhesion, degrade basement membrane, become fusiform and migrate into the surrounding mesenchyme. In the current study, we asked if nicotine treatment would affect epithelial-to-mesenchymal transformation during palate development in an in vitro mouse model. Embryonic (13.5 day) mouse palatal shelves were cultured in serum free media and treated with 0, 0.6 mM, or 6 mM nicotine hemisulfate. Tissues were harvested after 72 hours and processed for H&E and immunohistochemical analysis of laminin, a specific marker for basal lamina. The fate of midline epithelia was traced by carboxyfluorescence labeling and analyzed by confocal microscopy. In control cultured tissues, basal lamina was absent in the midline and mesenchyme achieved confluence after 72 hours. However, in the groups treated with nicotine, medial edge epithelia remained in the midline and laminin staining was positive in a dose dependent manner. In conclusion, our results demonstrate that nicotine inhibits epithelial-mesenchymal transformation during palatal fusion in vitro.

857.2

Utilization of the Zebrafish to Understand Hematopoiesis

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Several genes have been implicated in the differentiation and development of hematopoietic and vascular progenitor cells, yet our understanding of the discrete steps involved in the induction of these cells from the ventral mesoderm is still incomplete. The zebrafish (*Danio rerio*) is an especially robust vertebrate system to both isolate and characterize these processes. One strength of the zebrafish system lies in the ease in which hematopoietic mutants have been generated and studied. These mutants span many of the proposed steps of both the primitive and definitive hematopoietic programs. We have obtained 26 complementation groups of mutants with defects in the hematopoietic program, each representing distinct regulatory events in the process of stem cell induction, proliferation and/or differentiation. Several of these mutant genes have been cloned using candidate and positional cloning approaches. Some of the mutants represent zebrafish models of human diseases. For instance, the *sauternes* mutant phenotype is due to a defect in the orthologous gene that causes the human disease, congenital sideroblastic anemia. We have also isolated novel genes in the hematopoietic program, such as ferroportin 1, an iron transporter that exports maternal iron stores to the fetus in all vertebrates. A novel screen is also underway to find mutants defective in the expression of the hematopoietic transcription factor *scl*, a basic helix-loop transcription required for normal blood and blood vessel formation. We have also utilized transgenic zebrafish with cell-specific promoters driving green fluorescent to examine the development of the blood island. Through the analysis of these newly derived zebrafish mutants, we hope to develop a better understanding of normal hematopoiesis as well as disease.

857.3

Altered Expression of n-NOS in the Cerebellum of Calcium Channel Mutant Mice

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Tottering, Nagoya rolling, and leaner mice exhibit varying degrees of cerebellar ataxia. These mice each express a different autosomal recessive mutation in the alpha1A calcium ion channel subunit gene. These mutant mice are models for several human neurologic disorders: familial hemiplegic migraine, episodic ataxia type-2, and spinocerebellar ataxia type-6 which are all associated with mutations in the human alpha1A calcium ion channel subunit gene. Ultrastructural analysis of the cerebellum of these mutant mice revealed altered synaptic contacts between Purkinje cell dendritic spines and cerebellar granule cell parallel fiber varicosities. Nitric oxide (NO), is an important messenger molecule in the central nervous system, especially in the cerebellum. We examined NO expression indirectly in cerebella of these mutant mice using NADPH-diaphorase histochemical staining and in situ hybridization histochemistry (ISHH) with antisense n-NOS mRNA. n-NOS ISHH labeling was observed in the cerebellar granule cell layer and molecular layer including basket cells and satellite cells but not in Purkinje cells. n-NOS mRNA expression and NADPH-diaphorase histochemistry were elevated in the tottering and Nagoya rolling mouse cerebella but decreased in the leaner mouse cerebellum. These findings suggest that NO may act as a mediator in the neuropathology of these mutant mice. This work was supported by the Brain Korea 21 Project to I.J.R., and NIH grant K08NS01681 to L.C.A.

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Real-time imaging of lipid processing in wild type and mutant zebrafish

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The optical clarity of zebrafish (*Danio rerio*) larvae was exploited to visualize lipid processing in living fish and provide the basis for a mutagenesis screen. Intense gall bladder fluorescence was observed when larvae ingested fluorescent lipids. Two quenched BODIPY-labeled phospholipids that fluoresce or shift their wavelength of emission following phospholipase A_2 (PLA_2) cleavage were used to monitor lipid digestion in real-time. Gall bladder fluorescence was found to reflect lipid processing by intestinal PLA_2 and subsequent transport through the hepatobiliary system. Using these lipids in a pilot mutagenesis screen, a number of punitive mutations have been identified that have altered patterns of fluorescence. Preliminary analysis of one such mutation (*canola*) suggests that the affected gene regulates intestinal lipid processing since hepatobiliary function in this mutant is normal. We have also developed methods for delivering fluorescent cholesterol analogs to also visualize cholesterol metabolism. Fluorescent lipids provide a sensitive readout of digestive physiology in living animals and demonstrate the utility of zebrafish for the genetic analysis of vertebrate physiology.

857.5

Ultrastructural Evidence for Protection from Streptozotocin-Induced Damage in Pancreatic B Cells by Metallothionein Overexpression

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Streptozotocin (STZ) is a pancreatic B cell toxin that is believed to stimulate the release of reactive oxygen species leading ultimately to depletion of cellular NAD+ and cell destruction. Metallothionein (MT) is an inducible antioxidant protein that has been shown to protect several cell types from injury. We examined STZ-treated isolated pancreatic islets from normal (FVB) mice and a transgenic (HMT-1) line that specifically overexpresses MT in pancreatic B cells. TEM studies of untreated islets from both mouse lines showed that approximately 60-70% of the cross-sectional area was occupied by cells ultrastructurally indistinguishable from published reports of insulin-producing B cells. Following STZ treatment, B cells from normal FVB mice islets showed several features of necrosis including vacuolization, moth-eaten mitochondria, dilated cisternae of RER, and electron-lucent cytoplasm. Other apparent B cells demonstrated normal cytological features but exhibited various stages of degranulation. Overall, B cells in STZ-treated preparations showed fewer granules than their untreated counterparts and many were morphologically disorganized or frankly necrotic. Significantly, non-B endocrine cells were intact, highly granulated, and in all respects indistinguishable from the same cell types in untreated controls. In contrast, B cells in STZ-treated islets isolated from HMT-1 transgenic mice showed no evidence of necrosis, and though some cells were moderately degranulated, vacuolization and biomembrane discontinuities were not seen. In general, both B and non-B cells showed intact cytoarchitectures with numerous dense-cored granules, primarily of the α and β type, in a background of moderately dense cytoplasm. We conclude that MT prevents degranulation and B cell damage in STZ-treated islets, and that the induction of endogenous MT genes may provide an alternative strategy for B cell protection from cytopathological responses to reactive oxygen species.

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ZEBRAFISH: A GENETIC MODEL FOR VASCULAR OCCLUSION

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Virchow postulated that thrombosis occurs due to abnormalities in the properties of blood, vessel wall and blood flow. Despite extensive *in vitro* characterization of blood coagulation, the actual pathological process of thrombosis *in vivo* is still elusive. Current animal models of vascular occlusion have focused on the mechanisms of thrombus formation and lysis as well as the effects of pharmaceutical agents, but have not been used as a genetic screen for hypo- or hypercoagulable stages. In this abstract, we report on the use of zebrafish as a model to study *in vivo* vascular occlusion. Our laboratory has previously shown the relevance of zebrafish to mammalian hemostasis. We show that ferric chloride ($FeCl_3$) and phenylhydrazine (PHZ) cause vascular occlusion in zebrafish larvae and the time to occlusion (TTO) can be reliably and rapidly detected. Vascular occlusion was induced by $FeCl_3$ and PHZ in either the sinus venosus of the yolk sac or caudal artery depending on the developmental stage of the larvae. To demonstrate that the occlusive event is due to a clot formation, we have sectioned larvae after chemical treatment and found evidence for fibrin deposition and platelet activation. To use this assay as a genetic screen, we have generated gynogentic diploid embryos from Florida wild-type zebrafish by early pressure treatment of eggs fertilized with UV-treated sperm. Screening of these larvae have indentified several batches with significantly prolonged TTO. This constitutes the first embryonic screen for vascular occlussion in zebrafish and should be useful in the determination of plasmatic or cellular elements involved in *in vivo* vascular occlusion as well as the identification of novel genes involved in *in vivo* thrombosis formation.